Incidence of *Soybean dwarf virus* and Identification of Potential Vectors in Illinois

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ABSTRACT

Harrison, B., Steinlage, T. A., Domier, L. L., and D'Arcy, C. J. 2005. Incidence of *Soybean dwarf virus* and identification of potential vectors in Illinois. Plant Dis. 89:28-32.

Soybean dwarf virus (SbDV), which causes an important disease of soybeans in Japan, is persistently transmitted by aphids and is endemic in forage legumes in the United States. To determine the incidence of SbDV in Illinois, we collected clovers and forage legumes in a total of 49 Illinois counties in 2001 and 2002 and tested them for the presence of SbDV by reverse-transcription–polymerase chain reaction. SbDV was detected in 43% of red clover (*Trifolium pratense*), 10% of white clover (*T. repens*), and 3% of yellow sweet clover (*Melilotus officinalis*) plant samples. The dwarfing strain (SbDV-D) was the predominant strain detected in Illinois. In 2000, *Aphis glycines*, an aphid species that colonizes soybeans, was reported for the first time in North America. To determine whether *A. glycines* or aphid species found colonizing clover were vectors of SbDV, transmission studies were conducted. Aphids of the species *Nearctaphis bakeri* reproducibly vectored SbDV among red clovers, and from red clover to soybean. *A. glycines* did not transmit SbDV; neither did two other clover-infesting aphid species, *Acyrthosiphon pisum* and *Therioaphis trifolii*.

Soybean dwarf virus (SbDV), a member of the *Luteoviridae*, was first identified in association with outbreaks of dwarfed soybean (*Glycine max* L.) plants that had severe yield losses in northern Japan in 1969 (29). Subsequently, similar viruses were identified in Australia, Ethiopia, Iran, New Zealand, Syria, and the United States (1,20,22–25,33). In northern regions of Japan, SbDV is one of the most important virus diseases of soybean (15). Yield losses from SbDV have been shown to be linearly correlated with percent SbDV infection in soybean fields, with a 50% infection causing a 40% yield loss that results from a reduced number of pods set (2,28). Due to the persistent nature of its transmission, SbDV is transmitted efficiently only by colonizing aphids (26). In the United States, SbDV has been detected primarily in clover plants and rarely infects soybean, presumably because of the absence of aphid vectors that colonize soybean (9,10).

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Accepted for publication 26 July 2004.

DOI: 10.1094/PD-89-0028

In Japan, the polyphagous *Aulacorthum solani* (Kaltenbach) is the principal vector of SbDV (15). In northern Japan, *A. solani* is holocyclic, and its eggs overwinter on red clover (*Trifolium pratense* L.) and white clover (*T. repens* L.) plants, many of which are infected with SbDV (2,15). In the spring, viruliferous winged aphids develop on these hosts and fly to soybean, where feeding of viruliferous aphids leads to SbDV infections. The disease is spread in the field by parthenogenic apterous aphids throughout the summer, until sexual generations emerge again in autumn and migrate back to clover to lay eggs and complete their life cycle (2,15).

Many species from several plant families are susceptible to SbDV, but the most common hosts of SbDV are members of the Fabaceae (4,8,30). Based on the symptoms they produce in soybean, SbDV isolates have been divided into two strains: dwarfing (SbDV-D) and yellowing (SbDV-Y) (27). SbDV-D symptoms in soybean plants include shortened internodes and petioles and dark colored, brittle leaves that curl downward. SbDV-Y causes less stunting, but the symptoms tend to be more severe and include interveinal chlorosis, thickened and brittle mature leaves, and leaflets that do not fully develop and become rugose (30). Dwarfing and yellowing strains of SbDV show differences in host range. Dwarfing strains infect red clover but not white clover, while yellowing strains infect white clover but not red clover (8,26).

Like other members of the *Luteoviridae*, SbDV isolates can show differential transmission by different aphid species, which has been used to further subdivide the dwarfing and yellowing strains (32). SbDV-DS strains are transmitted by *A. solani*, and SbDV-DP strains are transmitted by *Acyrthosiphon pisum* (Harris) and *Nearctaphis bakeri* (Cowen). Similarly, SbDV-YS strains are transmitted by *A. solani*, and SbDV-YP strains are transmitted by *Acyrthosiphon pisum*, *N. bakeri,* and very rarely by *Aphis glycines* Matsumura (16). SbDV isolates from different geographic regions also can display different transmission specificities. Japanese SbDV isolates were transmitted by *A. solani, Acyrthosiphon pisum*, and *N. bakeri*, but not by *Myzus persicae* (Sulzer) or *Aphis craccivora* Koch (16,26). In New Zealand and Tasmania, *Acyrthosiphon pisum*, *Aulacorthum solani*, and *Macrosiphum euphorbiae* (Thomas) vectored white-clover infecting isolates of SbDV, but as in Japan, *M. persicae* and *Aphis craccivora* did not transmit SbDV (19). In the United States, SbDV isolates from white clover were transmitted by both *Acyrthosiphon pisum* and *M. persicae*, but not by *Aulacorthum solani* (9,12,19).

This specificity of transmission is engendered by interactions between aphid membrane barriers and virus proteins. Gildow et al. (12) examined the movement of transmissible and nontransmissible SbDV isolates through *M. persicae* and observed that a nontransmissible SbDV isolate could not penetrate the salivary basal lamina of *M. persicae*. The salivary basal plasmalemma, the cell layer adjacent to the basal lamina, served as the barrier for transmission for an isolate that was not transmitted by *A. solani*. SbDV-D and SbDV-Y strains share 83% amino acid sequence identity (32). The greatest sequence variability between the two strains of SbDV is found in the C-terminal half of the coat protein readthrough domain and the 3′ noncoding region (32). Yet amino acid sequence variation in the N-terminal region of the readthrough domain has been reported to be related to specificity of aphid transmission of SbDV (31).

Aphis glycines was identified in the United States for the first time in 2000 (13) and subsequently has spread throughout much of the soybean growing region of North America (17). The introduction of *A. glycines*, an aphid species that colonizes soybean plants, has the potential to increase the incidence in North America of persistently and nonpersistently transmitted viruses in soybean. *A. glycines* has

been reported to transmit several viruses, including *Alfalfa mosaic virus, Bean common mosaic virus, Bean yellow mosaic virus, Cucumber mosaic virus, Indonesian soybean dwarf virus* (ISDV), *Peanut stripe virus, Peanut stunt virus, Soybean mosaic virus* (SMV), and *Tobacco ringspot virus* (3,7,14,18). ISDV is another member of the *Luteoviridae* that causes symptoms similar to SbDV in soybean but is serologically distinct from SbDV (18). While in one instance *A. glycines* was reported to transmit SbDV rarely (15), other reports have found no transmission of Japanese isolates of SbDV by *A. glycines* (28,29).

This is the first study of SbDV in Illinois. Our objectives were to determine the distribution of SbDV in Illinois in forage legumes that border soybean fields, determine the prevalent strain(s) of the virus, and identify potential vectors of the virus in Illinois.

MATERIALS AND METHODS

Reverse transcription–polymerase chain reaction (RT-PCR). SbDV infections were detected by RT-PCR. RNA was extracted from leaf tissue homogenized in TRIZOL reagent (Invitrogen Corporation, Carlsbad, CA) following the manufacturer's recommendations. For RT-PCR, the SuperScript One Step RT-PCR System (Invitrogen) was used, and reactions were performed in PTC-100 Programmable Thermal Controllers (MJ Research, Inc., Watertown, MA). Reactions consisted of 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s. For most RT-PCR assays, SbDV-3248F (GGAACTATCACTTTCGGGCCGTCT) and SbDV-3529R (GGCATGATACCAGTGAAGACC) were used to amplify a 281-nucleotide (nt) fragment from the coat protein (CP) gene of SbDV. A degenerate primer pair was developed to test plants for the presence of other luteoviruses. The primers Dicot-Luteo 1F (TCACITTCGGGCCGWSTYTWTCAG) and Dicot-Luteo 1R (GGRTCIAIYTCRTAAGMGATSGAVCC) were designed to amplify a highly conserved region in the CP genes of the following dicot-infecting luteoviruses: *Bean leaf roll virus* (BLRV; GenBank accession no. AF441393), *Beet chlorosis virus* (AF352025), *Beet western yellows virus* (BWYV; AF473561), *Chickpea stunt disease associated virus* (CpSDaV; Y11530), *Cucurbit aphid-borne yellows virus* (X76931), *Groundnut rosette assistor virus* (GRAV; Z68894), *Potato leaf roll virus* (PLRV; AY138970), SbDV (L24049), and *Turnip yellows virus* (AF168606). The primers were tested in RT-PCR reactions with an annealing temperature of 60°C using 20 ng of purified BLRV, BWYV, *Barley yellow dwarf virus-RMV* (BYDV-RMV), *Cereal yellow dwarf virus-RPV* (CYDV-RPV), and PLRV. To differentiate dwarfing and yellowing SbDV strains, a primer pair (SbDV-5406 and SbDV-5493; Fig. 1) was synthesized that flanked a region in the 3′ NCR that was diagnostically variable between dwarfing and yellowing strains. RT-PCR reactions were performed as above with an annealing temperature of 55°C.

Incidence of SbDV infection. Clover and other plants were collected from ditches and field borders during the growing seasons of 2001 and 2002 from an average of two locations per county. In 2001, entire plants were dug up from 35 counties, brought back to the greenhouse, and replanted in pots. Plants that tested positive for SbDV by RT-PCR were used as sources of inoculum for transmission studies described below. In 2002, leaf tissue from clover plants was collected from an average of two locations in 36 Illinois counties. All plants were analyzed for SbDV infection using RT-PCR. In 2001, a subset of plants negative for SbDV infection with primers SbDV-3248F and SbDV-3529R were retested by RT-PCR using Dicot-Luteo 1F and Dicot-Luteo 1R. In 2002, the clover samples were analyzed only by RT-PCR with SbDV-3248F and SbDV-3529R.

Aphid colonies. Virus-free aphid colonies were established for five aphid species collected from Illinois: *Aphis craccivora, A. glycines*, *Acyrthosiphon pisum*, *N. bakeri*, and *Therioaphis trifolii* (Monell). Aphids were identified as described by Blackman and Eastop (3), and identifications were confirmed by David Voegtlin of the Illinois Natural History Survey. To clean the field-collected aphids of persistently transmitted viruses, they were placed on greenhouse-grown healthy red clover leaves and new-borne nymphs were immediately transferred to leaves of healthy plants. *Aphis craccivora* was reared on healthy *Vicia faba* L. cv. Improved Long Pod. *Aphis glycines* was reared on healthy soybean cv. Williams 82. *Acyrthosiphon pisum*, *N. bakeri*, and *T. trifolii* were reared on healthy red clover. All colonies were maintained in cylindrical acrylic cages in growth chambers maintained at 23°C and 17-h day length.

Transmission of SbDV by aphids. SbDV-D isolates were used for transmission studies because they were the most prevalent in Illinois. Five SbDV-positive clover samples, two from Champaign County and one each from Coles, Cumberland, and Douglas counties in Illinois, were used as source tissue in initial aphid transmission tests for SbDV-D. Preliminary experiments were performed to determine if *Acyrthosiphon pisum* (a potential positive control for SbDV-D transmission) or *Aphis glycines* transmitted SbDV-D when soybean seedlings were infested with multiple aphids. Approximately 20 aphids of *Acyrthosiphon pisum* and *Aphis glycines* were placed on detached leaves from red clover infected with each of the five SbDV isolates in petri dishes containing moistened filter paper and given an acquisition access period

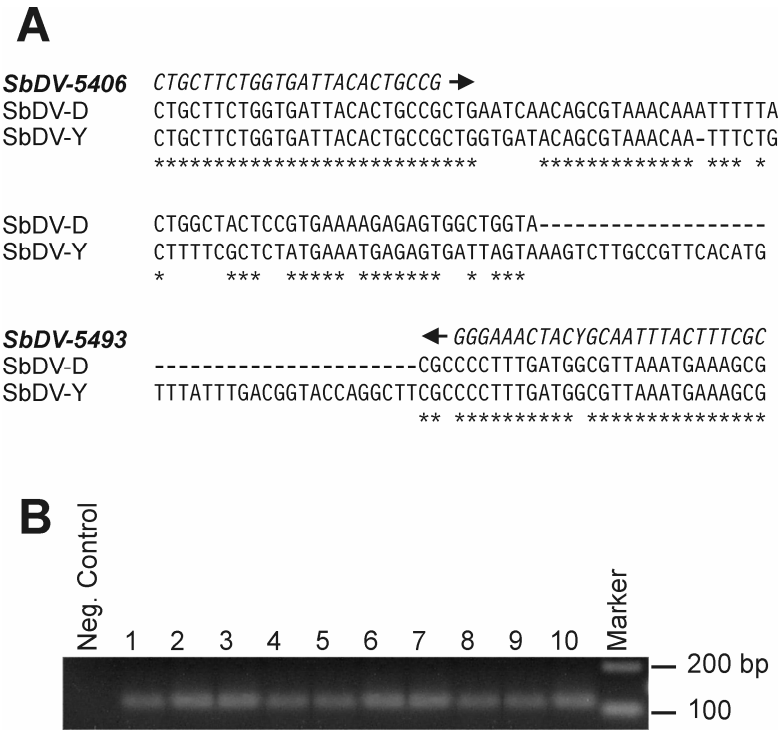

Fig. 1. Differentiation of *Soybean dwarf virus* (SbDV) strains. **A,** The sequences of primers SbDV-5406 and SbDV-5493 are shown above an alignment of the corresponding 3′ noncoding regions of SbDV-D and SbDV-Y strains. Amplifications of SbDV-D isolates are predicted to yield 110-bp fragments, while SbDV-Y isolates should produce fragments of 151 bp. **B,** Analysis of 10 Illinois SbDV isolates from red clover with primers SbDV-5406 and SbDV-5493. All samples analyzed produced 110-bp fragment, the size predicted for dwarfing isolates of SbDV.

(AAP) of 48 h. They were then transferred to two pots, each containing three healthy Williams 82 soybean seedlings at the unifoliate stage. After a 72-h inoculation access period (IAP), aphids were fumigated with Vapona (B.G. Pratt Co., Patterson, NJ). Three weeks after infestation, individual soybean plants were tested for SbDV infection by RT-PCR. The experiment was conducted twice. In total, 21, 18, 21, 21, and 18 soybean seedlings were infested with *A. glycines* that had fed on the SbDV-infected tissue from the two locations from Champaign County and the single locations from Coles, Cumberland, and Douglas counties, respectively. Similarly, a total of 18, 15, 18, 18, and 18 soybean seedlings were infested with *Acyrthosiphon pisum* that had fed on the SbDV-infected tissue from two locations from Champaign County and single locations in Coles, Cumberland, and Douglas counties, respectively.

To evaluate the vector competence of clover-infesting aphids endemic to Illinois, four species of aphids, *Aphis craccivora, Acyrthosiphon pisum*, *N. bakeri*, and *T. trifolii*, and *Aphis glycines* were tested for their abilities to transmit SbDV-D among red clover plants. Approximately 100 aphids of each species were placed on detached infected red clover leaves for 48 h and then transferred to two pots per species containing approximately 30 red clover seedlings, covered with cylindrical acrylic cages, sealed, and placed inside a growth chamber at 25°C, 17-h daylight, and shaded from direct light. After a 2-week IAP, plants were fumigated. Plants in each pot were divided into three 10-plant samples collected at 6 and 10 weeks postinoculation. Total RNA was extracted from each 10-plant sample and analyzed by RT-PCR. The experiment was repeated a second time, with some modifications. The AAP was as described above, but 60 aphids were used to inoculate each of two pots per aphid species containing five clover plants each. The aphids were killed after a 96-h IAP. Individual plants were sampled 3 weeks after infestation and tested by RT-PCR.

The same five aphid species, *Aphis craccivora, A. glycines, Acyrthosiphon pisum, N. bakeri,* and *T. trifolii,* were tested for transmission of SbDV from red clover to soybean. Aphids were given 48-h AAP on SbDV-D–infected red clover leaf tissue in petri dishes. For each aphid species, approximately 100 late instar aphids were transferred to pots containing three to four Williams 82 soybean plants at the unifoliate stage. After a 72-h IAP, the plants were fumigated. Plants were sampled individually and analyzed by RT-PCR 3 weeks after infestation.

To determine the efficiency of aphid transmission of SbDV from red clover to soybean, *Aphis glycines* and the two aphid species that vectored SbDV among red clovers, *Aphis craccivora* and *N. bakeri,* were analyzed further. The experiment was conducted twice, inoculating with either one or seven aphids per plant at 25 and 30°C. Aphids were given a 24-h AAP on detached leaves from SbDV-D–infected red clover plants in petri dishes in a growth chamber at 25 and 30°C, 17-h daylight, and shaded from direct light. Next, the aphids were transferred to unifoliate Williams 82 soybean seedlings. For single-aphid inoculations, the aphids were transferred to soybean seedlings and covered by a leaf cage. For inoculations with multiple aphids, trays containing 20 to 24 plants were covered by rectangular acrylic cages after transferring seven aphids to each plant. The trays with the aphid-infested plants were put inside a growth chamber at 25 or 30°C and 17-h daylight for 96 h. The plants were assayed for symptoms and tested by RT-PCR 3 weeks after infestation.

A final transmission study to test the ability of *Aphis glycines* to vector SbDV from infected to healthy soybean plants was conducted. Two SbDV-infected soybean plants were placed in separate 25 × 25 × 75 cm cages and infested with *A. glycines*. After 2 weeks, when the aphids densely colonized the SbDV-D–infected plants, pots containing five to six healthy soybean plants were introduced into the cages. After 4 weeks, when the introduced plants were heavily infested with aphids, all plants were tested by RT-PCR for SbDV infection.

RESULTS

Incidence of SbDV in Illinois. In 2001, 120 clover and other plants were collected from 35 Illinois counties and tested for SbDV (Fig. 2). Enzyme-linked immunosorbent assay using SbDV-specific monoclonal antibodies (11) was evaluated for detection of SbDV infections, but because of high backgrounds in clover samples (data not shown), RT-PCR was deemed more reliable and used to detect the presence of SbDV. Of the clover plants tested, 64 were red clover, 19 of which tested positive for SbDV by RT-PCR (Table 1). No SbDV infections were found in yellow sweet clover (*Melilotus officinalis* L.), black medic (*Medicago lupulina* L.), or white clover. To determine whether plants negative in RT-PCR with SbDV-3248F and SbDV-3529R were infected with other luteoviruses, the PCR-negative samples were reanalyzed by RT-PCR using degenerate primers Dicot-Luteo 1F and Dicot-Luteo 1R, which were designed from the sequences of dicot-infecting luteoviruses. All samples that were negative with the SbDV-specific primers (SbDV-

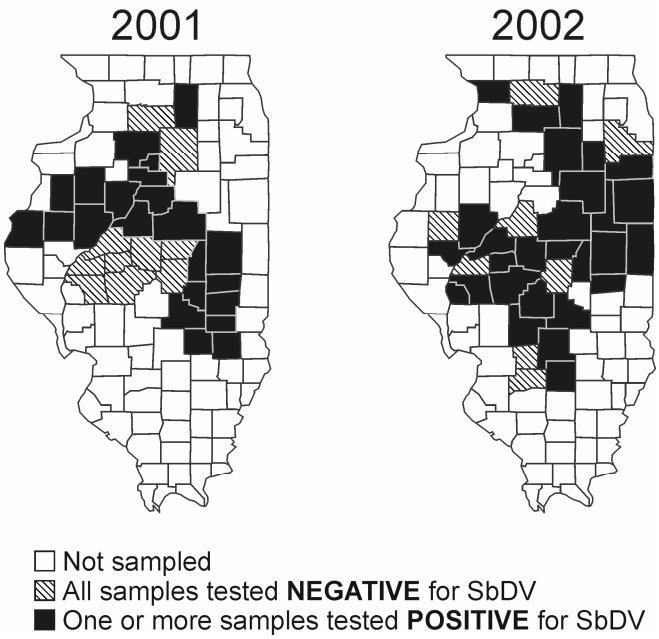

Fig. 2. Distribution of *Soybean dwarf virus* (SbDV) in clover and soybean plants in Illinois in 2001 and 2002. Maps depict distribution of SbDV-infected clover plants in Illinois counties in 2001 and 2002.

Table 1. Incidence of *Soybean dwarf virus* (SbDV) in forage legumes collected in Illinois in 2001 and 2002 as determined by reverse transcription–polymerase chain reaction (RT-PCR)

	SbDV positive	
Plant type	**2001**	**2002**
Red clover	19/64[z]	50/96
White clover	0/11	3/20
Yellow sweet clover	0/14	1/17
Black medic	0/17	0/20
Oxalis stricta	0/4	0/17
Other clover species	0/10	0/5
Total	19/120	54/175

[z] Number of plants positive by RT-PCR/number tested by RT-PCR.

3248F and SbDV-3529R) also were negative with the degenerate primers. As positive controls, the primers amplified fragments of the expected size from plants infected with SbDV and from purified preparations of BLRV, BWYV, BYDV-RMV, CYDV-RPV, and PLRV (Fig. 3). In 2002, 175 clover plants from 36 Illinois counties were sampled (Fig. 2) and tested by RT-PCR with SbDV-3248F and SbDV-3529R. From the 96 red clover samples, 50 tested positive by RT-PCR. Three white clover and one yellow sweet clover samples also were positive (Table 1).

Ten Illinois SbDV isolates from red clover were analyzed by RT-PCR with SbDV-5493 and SbDV-5406 to determine if they were yellowing or dwarfing strains. All 10 isolates tested produced bands of 110 nt as expected for dwarfing isolates rather than the 151-nt fragments expected from yellowing isolates (Fig. 1).

Aphid transmission of SbDV. In initial transmission studies using 20 aphids per plant, neither *Aphis glycines* (0 of 99 plants) nor *Acyrthosiphon pisum* (0 of 87 plants) transmitted SbDV-D from red clover to soybean. Since *A. pisum* was expected to serve as a positive control for the remaining transmission studies, three other aphid species found colonizing clover (*Aphis craccivora*, *N. bakeri*, and *T. trifolii*), along with *Aphis glycines* and *Acyrthosiphon pisum,* were tested for their abilities to vector SbDV-D. In transmission experiments using approximately 100 aphids per pot of red clover seedlings, two of three 10-plant samples from one pot inoculated with SbDV using *Aphis craccivora* were positive for SbDV, and one 10-plant sample from each of two pots inoculated with SbDV using *N. bakeri* was positive. SbDV was not transmitted by *Aphis glycines*, *Acyrthosiphon pisum,* or *T. trifolii* in this experiment. When the experiment was repeated with fewer clover plants and a shorter IAP, none of the aphid species transmitted SbDV from red clover to red clover. When the same five aphid species were tested for their abilities to vector SbDV-D from red clover to soybean using approximately 100 aphids of each species per pot of soybean seedlings, only *N. bakeri* transmitted SbDV.

Since *Aphis craccivora* and *N. bakeri* transmitted SbDV in preliminary tests, experiments were conducted to test the efficiencies of SbDV-D transmission from red clover to soybean by the two aphid species at 25 and 30°C. *Aphis glycines* was included in the trial as an additional test of its vector competence. When one aphid per plant was used, transmission of SbDV occurred only with *N. bakeri* at 30°C (Table 2). When the number of aphids per plant was increased to seven, again only *N. bakeri* transmitted SbDV-D from red clover to soybean (Table 2). The infected soybean plants showed symptoms characteristic of SbDV-D that included dwarfing due to shortened internodes and smaller, thicker, darker green and downward curled leaves, as described by Chang (6). Because of the low efficiency of transmission, it was not possible to draw conclusions regarding the influence of temperature on transmission. In the final transmission study, where *Aphis glycines* was allowed to completely colonize SbDV-D–infected soybean plants and then migrate to healthy soybean plants, no virus transmission was detected as determined by RT-PCR.

DISCUSSION

The dwarfing strains of SbDV were the most common in clovers in Illinois during 2001 and 2002. This conclusion was supported by RT-PCR results and by the predominance of infection of red clover, which is commonly infected by SbDV-D in both Japan and the United States but rarely infected by SbDV-Y (8,19,27). When only the SbDV-positive RT-PCR results are considered (Table 1), the detection of SbDV-infected red clover in Illinois increased from 30% in 2001 to 52% in 2002, with positive samples detected in eight additional Illinois counties (Fig. 2). While SbDV incidence may have increased in Illinois from 2001 to 2002, the larger area sampled and/or the improved sampling and detection techniques used in 2002 may have contributed to the increase in the number of positive plant samples.

In the present study, less than 10% of the white clover plants sampled were infected with SbDV. In contrast, Damsteegt et al. (9) detected SbDV in 47% of white clover samples from eastern and southeastern states. Several factors may be responsible for these contrasting results. The surveys in the eastern United States were conducted 5 years earlier in white clover pastures, whereas clovers in the current study were found in ditches surrounding soybean fields. Virus infection may be more likely in clover pastures where the plants are in a higher density and cover a larger area, conditions that favor disease spread. In addition, vegetative reproduction of white clover in pastures over multiple years can result in growth of several diseased plants originating from one infected parent. The dissimilar results also may be attributed to the different environments of the regions of the United States in which the studies were conducted, or to the use of different detection methodologies. Additional studies with different sampling protocols and possibly larger numbers of samples will need to be conducted to identify the source of the differences in incidences of SbDV-Y isolates in the two studies.

Acyrthosiphon pisum, *Aphis craccivora*, *N. bakeri*, and *T. trifolii* were found colonizing clovers in Illinois and tested for their ability to transmit SbDV. *Acyrthosiphon pisum* has been reported to vector U.S. isolates of SbDV (10). *Aphis craccivora* transmits at least 40 viruses, including the luteoviruses BLRV, BWYV, CpSDaV, and GRAV (5). In previous transmission studies in Japan and New Zealand, *A. craccivora* failed to transmit

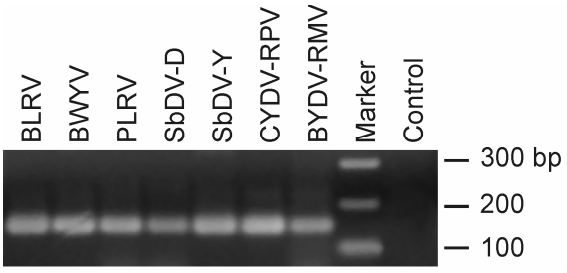

Fig. 3. Reverse transcription–polymerase chain reaction (RT-PCR) detection of members of the *Luteoviridae*. RT-PCR products produced by amplification of purified virus preparations of indicated viruses with Dicot-Luteo-1F and Dicot-Luteo-1R. All viruses analyzed amplified fragments of the expected 150 nt.

Table 2. Transmission of *Soybean dwarf virus* (SbDV-D) from red clover to soybean by three aphid species

	Transmission (one aphid per plant)				Transmission (seven aphids per plant)			
	Experiment 1		Experiment 2		Experiment 1		Experiment 2	
Aphid species	**25°C**	**30°C**	**25°C**	**30°C**	**25°C**	**30°C**	**25°C**	**30°C**
Aphis craccivora	0/24[z]	0/22	0/24	0/22	0/21	0/22	0/17	0/23
Aphis glycines	0/24	0/24	0/24	0/23	0/23	0/20	0/24	0/24
Nearctaphis bakeri	0/24	1/20	0/24	0/21	1/24	1/24	0/24	1/23

[z] Number of infected plants/number of inoculated plants.

SbDV isolates (26,33). *N. bakeri* colonizes red clover, but unlike *A. craccivora*, its potential as an aphid vector has not been studied in detail. However, it has been reported to transmit viruses in several families and was recently reported as a vector of SbDV-DP (3,16) and SbDV-YP (21). *T. trifolii* also has been reported to transmit legume-infecting viruses (3), but has not been reported to transmit SbDV. In our studies, *N. bakeri* transmitted SbDV in a few instances, but at very low efficiencies. *A. craccivora* transmitted SbDV in initial trials with large numbers of aphids (ca. 100), but did not vector SbDV when one or seven aphids were used per plant. These results may reflect events taking place in the field, i.e., low transmission rates by aphid species that colonize in large numbers, or there may be other aphid clones from these species that transmit SbDV-D more efficiently. The low transmission efficiencies observed in these studies also may have been due to low virus titers in the clover plants used as SbDV sources. It is also possible that other aphid species that were not collected and tested transmit SbDV at higher efficiencies.

As reported previously for Japanese SbDV isolates (28,29), we found no evidence of transmission of SbDV by *Aphis glycines*. Hence, *A. glycines* is unlikely to be an important vector of SbDV in Illinois. This is supported by the observations that none of the more than 1,000 soybean plants that were tested for SbDV in 2001 and 2002 were positive for SbDV infection despite high *A. glycines* populations (data not shown). *N. bakeri*, which was found heavily colonizing forage legumes close to soybean fields, was capable of transmitting SbDV-D to soybean plants, but only at low levels. In addition, *N. bakeri* produced very few nymphs on soybean plants that survived only a few days. These factors may explain why transmission of SbDV by *N. bakeri* has not become a significant threat to soybean production in Illinois.

ACKNOWLEDGMENTS

This work was supported by the United States Department of Agriculture–Agricultural Research Service and the Illinois Council for Food and Agricultural Research.

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